Review

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Optogenetic control of intracellular signaling pathways

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Cells employ a plethora of signaling pathways to make their life-and-death decisions. Extensive genetic, biochemical, and physiological studies have led to the accumulation of knowledge about signaling components and their interactions within signaling networks. These conventional approaches, although useful, lack the ability to control the spatial and temporal aspects of signaling processes. The recently emerged optogenetic tools open exciting opportunities by enabling signaling regulation with superior temporal and spatial resolution, easy delivery, rapid reversibility, fewer off-target side effects, and the ability to dissect complex signaling networks. Here we review recent achievements in using light to control intracellular signaling pathways and discuss future prospects for the field, including integration of new genetic approaches into optogenetics.

Challenges in accessing the dynamic information of intracellular signal transduction

Cells are constantly sensing and responding to extracellular stimuli in their environment. A central question in cell biology is how intracellular signaling pathways respond to the external environment to make appropriate decisions and how decision-making processes go awry in disease conditions. Genomics and proteomics have been continuously expanding our knowledge base of genes and proteins that are responsible for specific cellular functions. However, much less is known about the dynamic nature of signal mechanisms, primarily due to a lack of appropriate tools to access this dynamic information.

From an engineering viewpoint, intracellular signaling pathways serve as circuits for processing extracellular inputs, computing net results, and executing outputs. For instance, multiple signaling pathways are activated by growth factors (inputs) to regulate proliferation, differentiation, migration, and apoptosis (outputs). Intriguingly, distinct cellular outputs that are elicited by different growth factors often utilize the same set of intracellular signaling pathways [1]. It has been suggested that the output specificity is achieved by regulating intracellular signaling transduction in space and time. However, a better understanding of the spatiotemporal aspect is hindered by the technical challenges inherent in controlling specific signal cascades in space and time.

Conventional methods for studying signal transduction primarily involve pharmacological and genetic approaches. These approaches characterize cellular outputs in response to changes in certain signaling components elicited by chemical (agonist or antagonist) or genetic (gain- or loss-of-function mutations) perturbations. Such approaches proved to be crucial for identifying components involved in signaling pathways. However, these approaches lack the spatial and temporal control to decode the dynamic information in intracellular signal transduction. Chemical genetic approaches have been developed to improve the flexibility of signaling control by using chemical inducers to trigger the activation of engineered proteins [2,3]. Unfortunately, the diffusive nature of chemicals still hampers their capacity for precise spatiotemporal control.

Emerging optogenetic approaches (see Glossary) have led to novel ways of studying signal transduction in live biological systems. Initial successes in optogenetics used light to regulate neuroelectric activities and have transformed experimental neurobiology [4–8]. The field of optochemical control of cell signaling, which primarily uses photo-uncaging of small molecules [9–11] or unnatural amino acids [12–14] to trigger the activation states of signaling molecules, has also seen success. However, we focus on optical control of intracellular signaling pathways based on genetically encoded photoactivatable proteins.

Glossary

Association/dissociation time: the average time it takes to induce association or dissociation of photoactivatable proteins.
Association/dissociation wavelength: the wavelength of light used to stimulate the association or dissociation of photoactivatable proteins. Some photoactivatable proteins (such as CRY2 and LOV) do not have a light-driven dissociation mechanism. Instead, the complex dissociates spontaneously in darkness.
Cofactor: photosensitive small molecules bound to photoactivatable proteins. Cofactors are required for the photoactivation of photoactivatable proteins. Common cofactors include flavin (blue-light sensitive) and bilin (red-light sensitive) and their derivatives. Some photoactivatable proteins (such as UVR8 and Dronpa) do not have cofactors and use intrinsic amino acids such as tryptophan residues to mediate their conformational changes.
Optogenetics: combines the power of light and genetics and uses light-mediated protein–protein interactions to control the open/closed state of channels or the activation/inactivation states of signaling components within live cells.
Photoactivatable proteins: also referred to as photoreceptors. These proteins undergo light-induced conformational change to initiate signal transduction.
Photoexcitation: the process of converting photon energy to conformational changes of photoreceptors.

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In this type of optogenetic control, activities of intracellular signaling components are coupled to light-induced conformational changes of photoactivatable proteins [15–19]. We summarize current achievements in optogenetic control of signaling pathways, highlight advantages of precise spatiotemporal control, and explore future prospects.

Optogenetic control of cell signaling

Photoactivatable proteins

Photoactivatable proteins are core components of optogenetic control of intracellular signal transduction. Pioneering work by several research groups has led to the discovery of several photoactivatable proteins, such as light, oxygen, and voltage (LOV) domains [20–22], phytochrome B (PhyB) [23,24], cryptochrome 2 (CRY2) [25], UV-resistance locus 8 (UVR8) [26,27], and Dronpa [28] (Table 1 and Box 1). Some photoactivatable proteins, such as split GFPs [29,30] have yet to be used in controlling live-cell signal transduction, but there has been recent success in using light-controlled protein–protein interactions to regulate intracellular signaling pathways in live cells (Table 2). The mechanisms of these photoactivatable systems are well known [19]. By absorbing energy from the photons in excitation light, photoactivatable proteins undergo conformational changes, rearrange inter- or intraprotein contacts, and modulate inter- or intraprotein interactions (Figure 1). In general, optogenetic signaling control can be achieved by two general schemes: light-induced protein translocation and light-induced protein uncaging (Figure 2). In the protein-translocation scheme, interprotein interactions change the cellular location or the oligomerization state of signaling proteins, which can lead to downstream cellular responses (Figure 2 A–D). In the protein-uncaging scheme, signaling proteins are inactive until intraprotein interactions remove the steric block (Figure 2 E and F).

Mitogen-activated protein kinase (MAPK) signaling pathway

The MAPK signaling pathway plays important roles in controlling cell proliferation, differentiation, survival, and apoptosis. Light-controlled activation of the MAPK signaling pathway was first demonstrated in yeast by membrane recruitment of the scaffold protein Ste5, which was known to activate the MAPK pathway when tethered to the plasma membrane [31]. Ste5 was fused to a PDZ domain, which bound to a membrane-anchored LOV-epitope fusion protein on blue-light stimulation and subsequently activated the MAPK pathway. In mammalian cells, a light-induced MAPK (Ras/Raf/MEK/ERK module) activation system was built based on the PhyB–phytochrome-interacting factor (PIF) 6 system [32]. PhyB was anchored to the plasma membrane and PIF6 was fused to the catalytic segment of the protein SOS (SOS_{cat}). Red light induced PhyB–PIF6 binding and membrane recruitment of SOS, which subsequently activated the Ras/Raf/MEK/ERK signaling pathway. Light-controlled activation of the Raf/MEK/ERK pathway in mammalian cells has also been achieved by the cryptochrome-interacting basic helix–loop–helix (CIB1)–CRY2 system [33]. CIB1 was anchored to the plasma membrane and CRY2 was fused to Raf1. Blue-light stimulation recruited Raf1 to the plasma membrane, where Raf1 was activated to activate its downstream kinases. This approach used Raf1 as the controlling component to avoid potential crosstalk with other signaling pathways, which may be induced by upstream factors such as SOS. Light-induced activation of the Raf/MEK/ERK pathway stimulated significant neurite outgrowth in PC12 cells in the absence of nerve growth factors. Interestingly, neurite outgrowth did not require constant ERK activation. Intermittent on/off light control revealed a 45-min threshold for the light-off interval, which still supported maximum neurite outgrowth [33].

In addition to light-induced binding between CRY2 and CIB1, CRY2 has been shown to oligomerize on blue-light illumination [34]. Such a property allows light-induced aggregation of CRY2–Raf1 in the cytoplasm [35], which was able to activate Raf1 and the downstream Raf/MEK/ERK signaling pathway. It is worth nothing that heterodimerization between Raf1–CIB1 and Raf1–CRY2 in the cytoplasm did not induce ERK activation, probably due to steric effects that block Raf–Raf interaction.

Phosphatidylinositol (PtdIns) 3-kinase (PI3K) signaling pathway

PI3Ks phosphorylate the 3-hydroxyl group of PtdIns to produce signaling lipids, such as PtdIns 3,4,5-trisphosphate

Table 1. Characterization of individual light-sensitive protein pairs in optogenetic toolboxes

Photoactivatable protein	Size (amino acids)	Cofactor	Association/dissociation wavelength (nm)	Association/dissociation time	Refs
PhyB(FL)–PIF3	1211/524	PCB	650/750	s/s	[64,65]
PhyB(NT)–PIF3	621/524	PCB	650/750	s/s	[24]
PhyB–PIF6	908/100	PCB	650/750	ms/ms	[23,66]
CRY2–CIB1	612/335	FAD	450/dark	s/6 min	[67–69]
CRY2–CIB1	498/170	FAD	450/dark	s/6 min	[25]
CRY2–CRY2	498/498	FAD	450/dark	s/6 min	[34]
CRY2olig	498	FAD	450/dark	s/23 min	[47]
EL222 (LOV fast cycler)	150	FMN	450/dark	s/s	[70,71]
FKF1–G1 (LOV fast cycler)	619/1173	FMN	450/dark	min/h	[21]
LOVpep–ePDZ	153/194	FMN	450/dark	s/s	[22,31]
VVD–VVD (LOV slow cycler)	150/150	FAD	450/dark	s/s to days	[72,73]
Dronpa–Dronpa	257/257	None	400/500	s/s	[16,28]
UVR8–COP1C340	440/340	None	Dark/290–310	1–4 h/s	[26,74]
UVR8–UVR8	440/440	None	Dark/280–315	2–24 h/s	[27,75–77]

Box 1. Photoactivatable proteins

Photoactivatable proteins or photoreceptors are core components of the optogenetic control of intracellular signal transduction.

LOV domain

LOV is a small domain (125 amino acids) with a PAS (PER–ARNT–SIM) core that binds flavin mononucleotide (FMN), an endogenous cofactor that is ubiquitously produced by mammalian cells [70]. No external cofactors are needed when it is used in mammalian cells. Blue light photoactivates the LOV domain by inducing formation of a covalent adduct between FMN and the Sγ sulfur on a conserved cysteine residue, which causes the C-terminal Jα alpha helix to swing out from the LOV core domain. Hydrolysis of this cysteinyl–flavin bond in darkness returns the LOV domain to the ground state [71].

PhyB–PIF

Phytochromes are signaling photoreceptors with five identified members (PhyA through PhyE) that mediate many light-sensitive processes in plants, such as seed germination, seedling de-etiolation, and shade avoidance [78,79]. PhyB responds to red and IR light through reversible conformational change induced by photoisomerization of a covalently bound chromophore, PCB. The inactive form (Pr) changes into the active form (Pfr) after being exited with 650-nm light; Pfr, which initiates biological responses, can be converted back to Pr by absorbing IR light at 750 nm. A disadvantage of the PhyB–PIF protein pair is that its function requires the exogenous cofactor PCB.

Cryptochromes

Cryptochromes are involved in light-regulated cell elongation and photoperiodic flowering in *Arabidopsis thaliana*. Cryptochrome 2 (CRY2) binds to CIB1 under blue light and the CRY2–CIB1 complex dissociates in darkness. Interestingly, CRY2 has also been found to undergo blue-light-induced protein oligomerization [34], enabling applications that use a single protein to control signal transduction. A mutant of CRY2 (E490G or 'CRY2olig') that significantly enhances oligomerization was identified recently by a yeast two-hybrid assay [47]. Both full-length CRY2 (612 amino acids) and its N-terminal photolyase homology region (CRY2PHR, amino acids 1–498) can bind to either full-length CIB1 (335 amino acids) or CIBN (amino acids 1–170 of CIB1). For simplicity, we use the term CRY2–CIB1 to cover all combinations of CRY2 and CIB1 interactions.

UVR8

Unlike other photoreceptors such as LOV, phytochrome, and cryptochrome, UVR8 has no small-molecule cofactors and uses tryptophan residues as light-perception elements. In the absence of UV light, UVR8 forms a homodimer. UV light can induce disruption of cation-π interactions between tryptophan and arginine residues at the homodimer interface, resulting in instantaneous dissociation of the homodimer [74]. UVR8 monomer can bind to Constitutively Photomorphogenic 1 (COP1) [26]. Redimerization of the UVR8 monomer takes several hours *in vitro* [76,77]. The rate of redimerization appears much faster in plants, possibly mediated by Repressor of UV-B Photomorphogenesis (RUP) 1 and 2 [75].

Dronpa

The fluorescent protein Dronpa forms a tetramer under blue light. On cyan-light (500 nm) stimulation, the tetramer dissociates into monomers [28]. Like UVR8, Dronpa has no small-molecule cofactors and uses tryptophan for light sensing. The association/dissociation reaction is reversible.

(PIP3), that activate downstream pathways such as AKT, Rac/actin, and protein kinase C (PKC) to regulate diverse biological functions including cell growth, survival, migration, and cell cycle progression [36,37]. Light-controlled activation of the PI3K/PIP3 pathway has been achieved by a membrane recruitment assay based on PIF6–PhyB [38] in which PhyB was anchored to the plasma membrane and PIF6 was fused to the SH2 domain of a PI3K-binding protein, p85α. Red light induced PhyB–PIF6 binding and recruited the SH2 domain to the plasma membrane. Membrane-bound SH2 recruited PI3K to the membrane and led to the consequential production of PIP3. A similar scheme for controlling PI3K activity using the CIB1–CRY2 protein pair was reported recently [39]. Local photoactivation of PI3K in the growth cone of mouse hippocampal neurons induced growth cone expansion and increased the numbers of filopodia and lamellipodia. The CIB1–CRY2 protein pair has also been used to control phosphoinositide metabolism [40]. Whereas membrane recruitment of PI3K produced PIP3, membrane recruitment of a phosphatase resulted in dephosphorylation of PIP3. Local activation of the phosphatase caused loss of membrane ruffling as well as collapse and retraction of the cell edge.

Rho family GTPase activity

The Rho family of GTPase is a subfamily of the Ras superfamily and plays a role in regulating actin dynamics, cell mobility, and organelle development. Three Rho family members – Rac1, Cdc42, and RhoA – have been well studied. Light-controlled activation of Rho GTPases was initiated by light-controlled membrane recruitment of their guanine nucleotide exchange factors (GEFs) [23]. PhyB was anchored to the plasma membrane and PIF was fused to various GEFs including Tiam (Rac GEF), intersectin (Cdc42 GEF), and Tim (Rho GEF). Global recruitment of Tiam and intersectin generated lamellipodia and filopodia, whereas global membrane recruitment of Tim induced cell-body contraction. Subcellular local activation of Tiam enabled directed extrusion of lamellipodia in live 3T3 cells. Rho GTPase activity has also been controlled in a translocation assay by the FKF1–GI [21] and LOVpep–ePDZ systems [22,31].

In addition to the light-induced protein translocation methods, Rho family GTPase activity has also been controlled optogenetically by a LOV-based photo-uncaging system. In this case, a photoactivatable Rac1 was constructed by fusing the LOV2-Jα sequence to the amino terminus of a constitutively active Rac1 [20]. In the dark state, the LOV domain sterically blocked the effector binding site of Rac1; light-induced unwinding of the Jα helix released the steric inhibition and activated Rac1.

Receptor tyrosine kinase activity

The Ras/Raf/ERK, PI3K, and phospholipase C (PLC) signaling pathways are downstream of receptor tyrosine kinase activation. Blue light can be used to control the activity of fibroblast growth factor receptor (FGFR) and activate all three downstream signaling pathways through blue-light-induced CRY2 oligomerization. When a CRY2 domain was fused between a membrane-targeting sequence and the cytosolic catalytic domain of FGFR, light-induced oligomerization led to autoactivation of the engineered receptor and subsequent activation of all downstream signaling pathways [41]. In another example, a chimeric receptor was made by linking the LOV domain of

Table 2. Current applications of optogenetic control of signaling pathways

Cellular function	Photoactivatable protein	Controlling mechanism	Signaling protein	Model system	Refs
MAPK	LOV	Translocation	Ste5	Yeast	[31]
Ras/ERK	PhyB–PIF6	Translocation	SOS_{cat}	Mammalian	[32]
Raf/ERK	CRY2–CIB1	Translocation	Raf1	Mammalian	[33]
Raf/ERK	CRY2–CRY2	Translocation	Raf1	Mammalian	[35]
Phosphatase	CRY2–CIB1	Translocation	5-Phosphatases	Mammalian	[40]
PI3K	CRY2–CIB1	Translocation	SH2 of p85α	Mammalian	[40]
PIP3	CRY2–CIB1	Translocation	SH2 of p85β	Neuron	[39]
Rho GTPase	PhyB–PIF6	Translocation	Tiam, Tim, intersectin	Mammalian	[23]
Rho GTPase	LOV	Uncaging	Rac1	Mammalian	[20]
Rho GTPase	FKF1–G1	Translocation	Rac1	Mammalian	[21]
Rho GTPase	LOVpep–ePDZ	Translocation	Cdc24	Yeast	[31]
Rho GTPase	Dronpa	Uncaging	Intersectin	Mammalian	[28]
RTK	CRY2–CRY2	Translocation	FGFR	Mammalian	[41]
RTK	LOV	Translocation	FGFR	Mammalian	[42]
Protease	Dronpa	Uncaging	NS3-4A protease	Mammalian	[28]
Apoptosis	LOV	Uncaging	Caspase-7	Mammalian	[43]
Protein splicing	PhyB–PIF3	Translocation	Vacuolar ATPase (VMA) intein	Yeast	[45]
Protein secretion	UVR8	Dissociation	VSVG-YFP	Mammalian, neuron	[27]
Protein inactivation	CRY2–CIB1-MP	Translocation	Vav2, Tiam1, Rac1, Cdc42, RhoG, V_HH antibody	Mammalian	[46]
Protein inactivation	CRY2(E490G)	Translocation	CLC	Mammalian	[47]
Actin polymerization	CRY2(E490G)	Translocation	Nck, verprolin homology, central, acidic (VCA) domain of N-WASP	Mammalian	[47]
Protein degradation	LOV	Uncaging	Degron	Mammalian	[49]
Protein degradation	LOV	Uncaging	Degron	Mammalian, zebrafish	[50]
DNA transcription	PhyB–PIF3	Translocation	GBD/GAD	Yeast	[24]
DNA transcription	PhyB–PIF6	Translocation	GBD/GAD	Yeast	[52]
DNA transcription	CRY2–CIB1	Translocation	Split-Cre recombinase	Mammalian	[25]
DNA transcription	CRY2–CIB1	Translocation	Split-Cre recombinase	*Drosophila*	[57]
DNA transcription	CRY2–CIB1	Translocation	LexA/VP16	Yeast	[52]
DNA transcription	CRY2–CIB1	Translocation	GBD/GAD	Zebrafish	[53]
DNA transcription	FKF1–G1	Translocation	GBD/VP16	Mammalian	[21]
DNA transcription	FKF1–G1	Translocation	Zinc-finger protein (ZFP)/VP16	Mammalian	[55]
DNA transcription	EL222	Uncaging	Helix–turn–helix (HTH)/VP16	T cell, zebrafish	[54]
DNA transcription	UVR8–COP1	Translocation	Nuclear factor kappa B (NF-κB) activation domain/GBD	Mammalian	[26]
Endogenous DNA transcription	CRY2–CIB1	Translocation	TALE DNA binding/VP64	Mammalian	[61]

Aureochrome 1 from *Vaucheria frigida* to the intracellular catalytic domain of murine FGFR1 (Opto-mFGFR1) [42]. Photoactivated LOV domains dimerized and activated Opto-mFGFR1, which activated downstream ERK, AKT, and PLCγ signaling pathways.

Light-induced programmed cell death

Programmed cell death, or apoptosis, is a regulated cellular suicide mechanism. Caspases, a family of cysteine proteases, are critical regulators of programmed cell death. Once activated, initial caspases cleave and activate downstream effector caspases, which in turn degrade other cellular proteins and cause apoptosis. A light-controlled caspase has been constructed by fusing a LOV domain to the apoptosis-executing domain of caspase-7. Under dark conditions, the LOV domain caged the caspase activity; blue-light illumination induced conformational changes in the LOV domain and released the caspase activity, causing cell apoptosis within 1 h [43]. This method can potentially be used to induce cell apoptosis of non-transfected cancer cells. At low pH (<6.0), membrane fusion formed syncytia (cells with multiple nuclei) from engineered and cocultured cancer cells; subsequent illumination induced cellular apoptosis of syncytia and thereby the targeted cancer cells [44].

Light-controlled production, inactivation, and degradation of proteins

The ability to rapidly induce the production, inactivation, or degradation of specific proteins is imperative for dissecting complex signaling networks and identifying novel therapeutic targets. A light-inducible protein-production system has been constructed through protein splicing in yeast [45]. Protein splicing is a post-translational modification that can generate new proteins by removing an internal segment (intein) followed by ligation of the remaining N- and C-terminal segments (extein). In the light-inducible protein-splicing system, protein fragments were fused to two halves of an intein. Maltose-binding protein (MBP) was fused to intein-N (I^N) and Flag was fused to intein-C (I^C). The two epitopes MBP-I^N and Flag-I^C were then fused to PIF3 and PhyB, respectively.

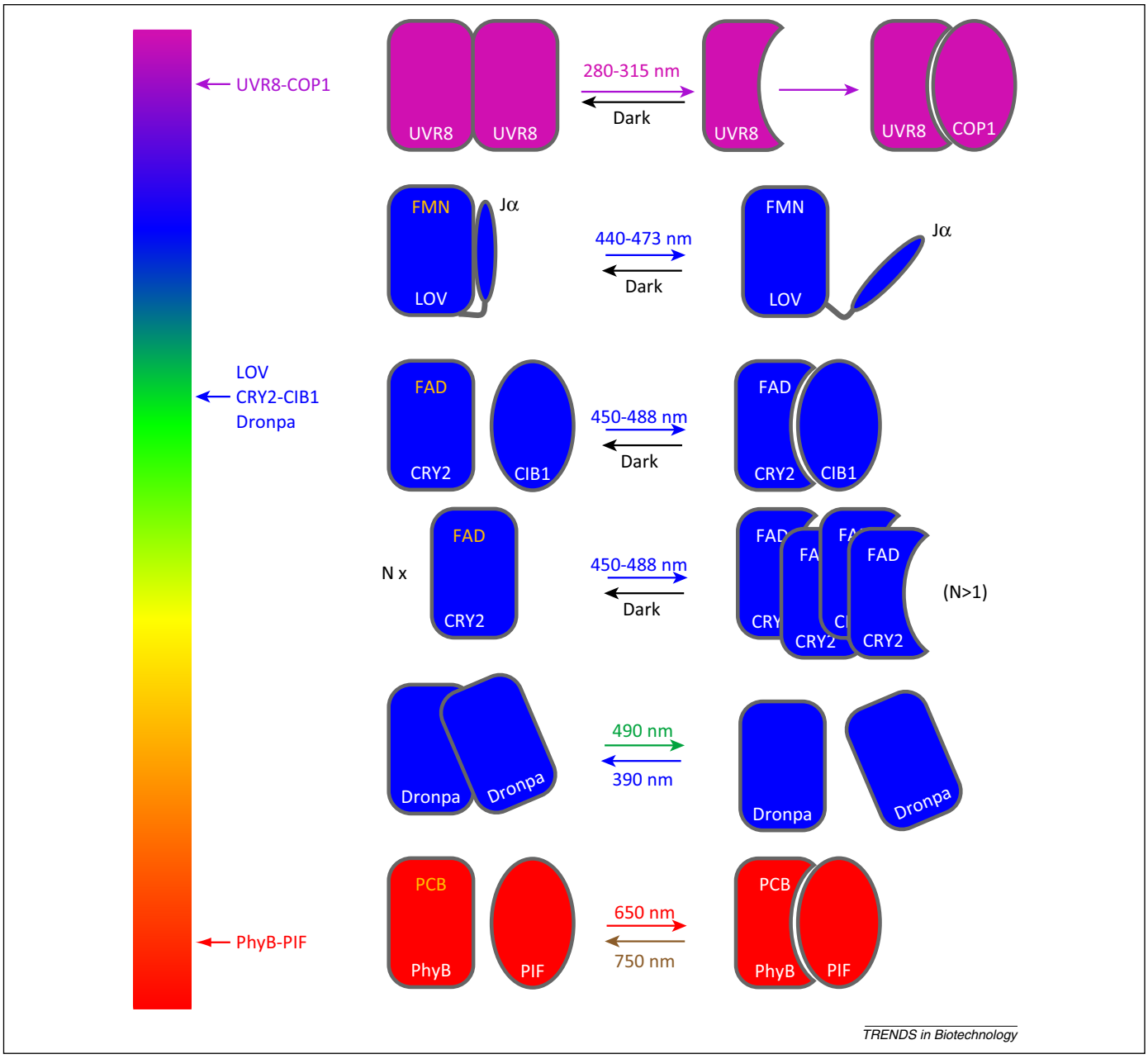

Figure 1. Scheme of light-induced conformational change in various photoactivatable proteins. The left bar illustrates the color of light (wavelength) that is used to stimulate photoactivation. Various protein pairs are shown on the right with light-induced intermolecular change [UV-resistance locus 8 (UVR8), cryptochrome 2 (CRY2)–cryptochrome-interacting basic helix–loop–helix (CIB1), CRY2 alone, and phytochrome B (PhyB)–phytochrome-interacting factor (PIF)] or intramolecular change [light, oxygen, and voltage (LOV), Dronpa]. For proteins containing cofactors [flavin mononucleotide (FMN), FAD, PCB], yellow marks the ground state and white marks the photoactivated state. UVR8 and Dronpa do not have cofactors and primarily use their tryptophan residues for photoreception.

PhyB–PIF3 binding on red-light illumination reconstituted the full-length intein, which autocatalytically excised itself to produce the splice product MBP-Flag. Such an experiment implies that light can be used to control the activity of proteins at the post-translational level in live cells.

A light-induced clustering strategy has been recently developed to inactivate target proteins [46]. The system uses both light-mediated heterodimerization of CRY2 and CIB1 and light-mediated homodimerization of CRY2. CIB1 was fused to a multimeric protein Ca^{2+}/calmodulin-dependent protein kinase IIα (CIB1-MP). The target protein was fused to CRY2. Photoactivated CRY2 proteins simultaneously oligomerized and bound to CIB1-MP, which induced the formation of higher-order clusters by promoting interconnection among MPs. Such clusters served as traps to inactivate target proteins including various GEFs (Vav2 and Tiam1) and GTPases (Rac1, Cdc42, and RhoG) [46]. Although CRY2 homo-oligomerization has been shown on photoactivation, hetero-oligomerization between CRY2 and CIB1-MP is significantly enhanced. A CRY2 mutant (E490G or 'CRY2olig') with a significantly improved photoinduced clustering effect was recently identified [47]. This mutation resulted in clustering of approximately 40–90% of cytosolic CRY2olig in 100% cells illuminated, compared with clustering of approximately 6% of cytosolic wild type CRY2 in around 12% of cells illuminated. Photoactivation of the fusion protein CRY2olig–clathrin light chain (CLC) resulted in enhanced transferrin uptake via

endocytosis. Clustering of Nck SH3 domains induced localized actin polymerization.

In addition to light-induced protein production and inactivation, light has also been used to induce protein degradation. Degrons, or destabilizing sequences, are small peptide sequences that can be recognized by specific proteasomes for protein degradation in live cells [48]. A light-controlled protein-degradation system has been constructed by fusing a 23-amino acid degron sequence to the C terminus of LOV2. On blue-light illumination, the normally caged degron sequence was exposed, resulting in rapid degradation of the fusion protein [49]. In another example, a smaller degron (five amino acids) was fused to the C terminus of the LOV domain. The LOV–degron was in turn fused to the target proteins (YFP, mCherry, and β-actin-mCherry) for light-induced degradation [50]. In both groups' work, photo-uncaging of LOV domains resulted in exposure of degrons to cause protein degradation.

Light-controlled protein trafficking and secretion

After being synthesized by ribosomes, proteins that are targeted for secretion are translocated into the endoplasmic reticulum (ER) to be glycosylated and correctly folded. If proteins aggregate within the ER, they are retained within the ER until the aggregates dissociate, and this phenomenon has been used in a ligand-induced protein-secretion system [51]. By fusing two or three copies of UVR8 to the C-terminal intracellular domain of vesicular stomatitis virus glycoprotein (VSVG), a light-induced protein-secretion system has been engineered [27]. In the dark state, UVR8s formed oligomers and VSVG-YFP–UVR8 was trapped in the ER. One 7-s pulse of UV light was sufficient to dissociate the VSVG-YFP–UVR8 clusters and induce protein trafficking from the ER to the Golgi complex and to the plasma membrane via the secretory pathway.

Light-controlled DNA transcription

Light-induced protein–protein interaction has been used to recruit transcription activators to promoters and subsequently regulate transcription of specific genes. The first implementations utilized the PhyB–PIF3 system. Two chimeric proteins – PhyB fused to GAL4 DNA-binding domains (GBDs) (PhyB–GBD) and PIF3 fused to GAL4-activation domains (GADs) (PIF3–GAD) – were expressed in yeast cells. PhyB–PIF3 binding on red-light illumination brought GBD and GAD into close proximity, which activated transcription of marker genes containing promoters with a GAL4 DNA-binding site. Gene expression can be repressed by far-red-light illumination, which dissociates the GBD–GAD complex [24]. Similar systems have also been constructed using the PhyB–PIF6 pair [52], the CRY2–CIB1 pair [52,53], and the LOV2 domain [21,54,55]. A different strategy used light-activated Cre recombinase to regulate DNA transcription [25] based on a split-Cre recombinase assay [56]. CRY2 and CIB1 were fused to the N and C domains of Cre, which can be reconstituted by photoactivation-induced CRY2–CIB1 association. Reconstitution of split Cre recovered its recombinase activity and activated the transcription of a reporter gene (*EGFP* preceded by a transcriptional stop sequence flanked by *loxP* sites). This light-induced split-Cre system has been used recently in *Drosophila* [57]. Various protein pairs have also been used in light-inducible systems to control mammalian cell gene expression [58].

Spatial and temporal control of intracellular signaling transduction

Light-controlled intracellular signal transduction is blessed with a unique capacity for spatial and temporal resolution. Light can be turned on or off instantaneously and can be directed to specific subcellular locations. Here we present a few examples to illustrate the powerful spatiotemporal control of light-activated intracellular signaling pathways.

Spatial control of subcellular signal activation

By activating a specific signaling pathway in a subcellular region, one can understand how localized signaling activation affects cell behavior. The most striking example is the localized activation of the Rho family GTPase that controls actin dynamics. Several studies have demonstrated the light control of Rac1, Cdc42, and RhoA [20,21,23,28]. In one case, local photoactivation of the LOV-based photoactivatable Rac1 (PA-Rac1) allowed reversible induction of membrane ruffles and protrusions [20]. Interestingly, activating Rac1 in one location near the cell edge also produced retraction on the opposite side of the cell. Other experiments using a protein-translocation assay [21,23] and an uncaging assay [28] also showed that localized activation of the Rho GTPase activity was sufficient to establish cell polarity. The process was associated with actin polymerization, translocation of downstream effectors, and localized PAK phosphorylation. Localized activation of FGFR signaling at the edge of a cell also resulted in cell polarity [41]. The PI3K signaling pathway was found to be primarily responsible for such an effect, consistent with previous findings that PI3K is an upstream activator of Rac1 [59].

The spatial resolution of optogenetic control may be degraded by the diffusion of activated signaling components. Using a PhyB–PIF6 system, such degradation in resolution can be rescued by deactivating the active species in the undesired areas [23]. In such a configuration, the activation can be confined to a highly localized area. This scheme is not necessary for the LOV domain where dissociation is fast, which limits the existence of activated signaling components in unilluminated areas. However, when the dissociation is slow (CRY2–CIB1 and UVR8) or when the binding is not reversible (VVD), the spatial resolution of localized signaling in cells is expected to be lower than what the light can achieve.

Temporal control of intracellular signaling pathways

How fast can cells catch up with changes in their environment? This timescale determines the adaptive capacity of cells. To understand this adaptive capacity, it would be ideal to stimulate cells at various frequencies and characterize the cellular responses accordingly. Light exposure can be precisely controlled in frequency and duration, enabling the measurement of the frequency of cell response. A PhyB–PIF6-based system was used to measure

how fast ERK activity caught up with the intermittent light stimulation [32]. It was found that the Ras/ERK module acts as a wide-bandwidth, low-pass filter, transmitting signals at a timescale of 4 min to 2 h.

On longer timescales, light activation of the Raf/MEK/ERK signaling module is sufficient to induce PC12 cell differentiation in 1–3 days [33]. A series of on/off light patterns was used to activate the Raf/MEK/ERK pathway in PC12 cells and revealed that the duration of the light-off dark interval was more crucial in determining the neurite length than the light-on duration. When the dark interval was equal to or less than 45 min, regardless of the light-on duration, the neurite length was the same as under continuous light illumination. By contrast, when the dark duration was longer than 45 min the neurite length decreased considerably. These studies demonstrate that light is a powerful tool to dissect the kinetic responses of intracellular signaling pathways.

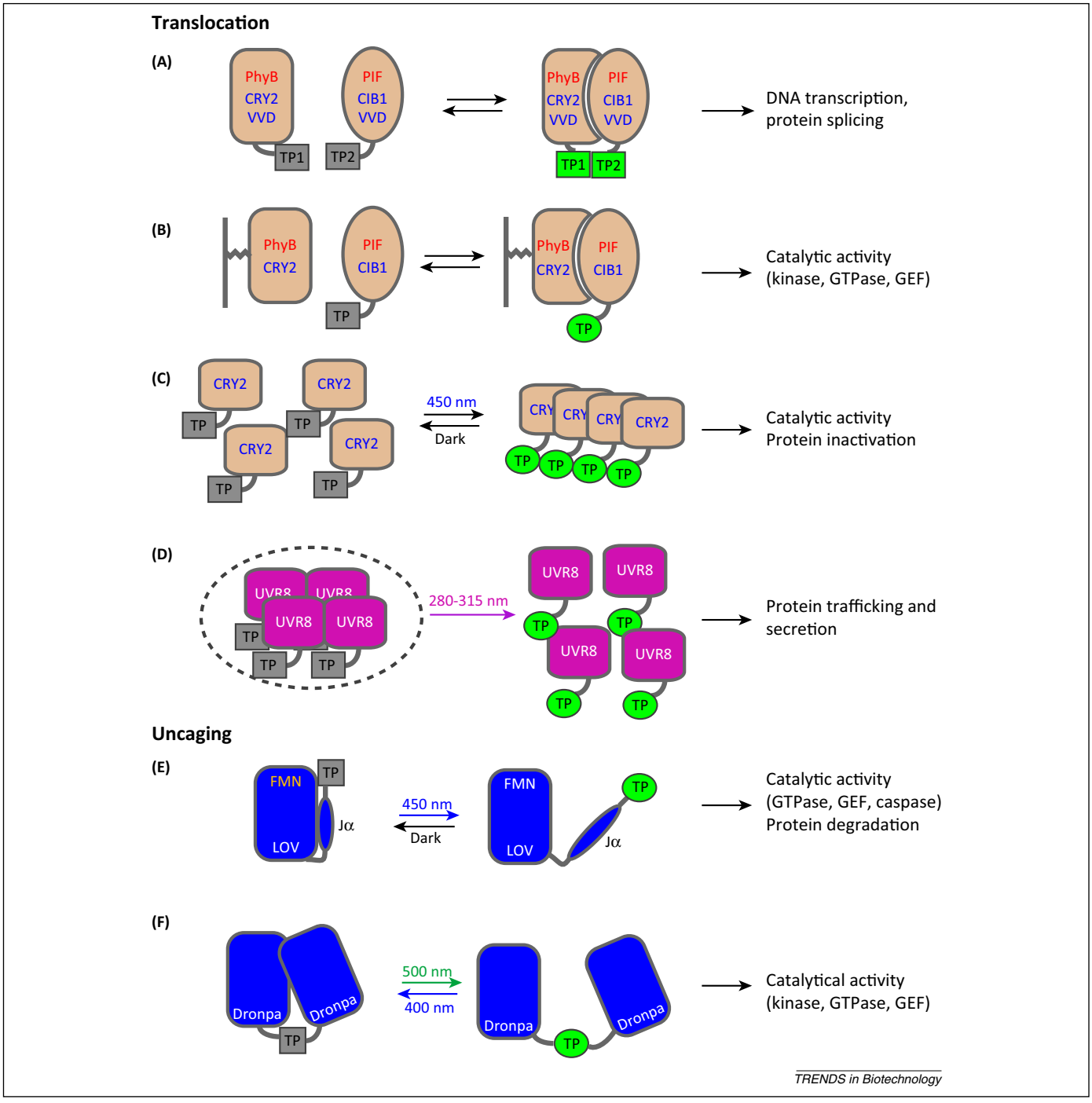

Figure 2. Modes of signaling control by photoactivatable proteins. The target proteins (TPs) can be activated by either photoinduced protein translocation (A–D) or uncaging (E,F). **(A)** Binding between TP1 (e.g., DNA-binding domain) and TP2 (e.g., activation domain) can lead to activation of DNA transcription. TP1 and TP2 can also be split inteins, which leads to protein splicing after protein binding. **(B)** Light can recruit signaling proteins to certain subcellular locations (e.g., the cytoplasmic leaflet of the plasma membrane) and activate downstream signaling pathways. **(C)** Light-induced oligomerization of CRY2 allows an increase in the local concentration of signaling protein (e.g., receptor tyrosine kinase) and subsequent activation of downstream pathways. Oligomerization can also be used to conditionally inactivate protein activities. **(D)** The oligomeric states of UV-resistance locus 8 (UVR8) can be used to trap or release proteins from organelles [e.g., the endoplasmic reticulum (ER)]. **(E,F)** Photo-uncaging can release the steric inhibition of signaling components and activate downstream pathways.

Precautions in designing optogenetic control of intracellular signaling

Some precautions should be used, however, in designing experimental schemes with optogenetic tools. Many photoreceptors, such as full-length phytochrome and cryptochrome, undergo light-mediated oligomerization. Oligomerization may orient the signaling components into configurations that differ from those that arise from naturally occurring ligand-induced dimerization. Such a difference may lead to differential outputs of subsequent signaling pathways. Additionally, basal signaling activities should always be checked in both the translocation and uncaging assays. In the translocation assay, overexpression of the optogenetic protein may lead to false binding; in the uncaging assay, basal activities may arise from imperfect allosteric inhibition. Screening and protein engineering are often needed for optimized performance. Negative controls, such as using dark conditions and activity-dead mutants, should be performed. The excitation light may also induce phototoxicity, depending on the wavelength, exposure time, and intensity, especially in applications that require long-term illumination. In such cases, protein pairs that can be activated by light with long wavelengths and low intensities would be preferred.

Concluding remarks and future perspectives

Recently developed genome-engineering techniques enable precise editing (deletion, insertion, or mutation) of genome sequences in mammalian cells [60]. Current photoactivatable proteins can be combined with these new genome-engineering tools to control endogenous gene transcription. One such integration has been demonstrated by the engineering of light-inducible transcriptional effectors (LITEs), in which a transcription activator-like effector (TALE) DNA-binding domain was fused to CRY2 and an effector (VP16) was fused to CIB1 [61]. Light-induced binding between CRY2 and CIB1 activated endogenous gene (*Neurog2*) transcription in mammalian cells. Such a technique can be generalized for precise regulation of gene expression and epigenetic states. Optogenetics could also extend its application in synthetic biology. The power of dynamically modulating signaling activity offers guidance for the design of customized signaling circuits [62]. As the optogenetic control of neuronal activity has already spearheaded translational research [63], the optogenetic control of intracellular signaling could potentially serve as a powerful tool complementing current treatment procedures, such as gene therapy, in clinical settings (Box 2).

Box 2. Outstanding questions

- How can we improve the photophysical properties of photoactivatable proteins, such as tunable activation wavelength, sensitivity, and dynamic range in response to the stimulating light, association and dissociation kinetics, and light-dependent oligomerization?
- Can orthogonal optogenetic tools be developed for simultaneous control of multiple signaling pathways?
- What is required to streamline the design and characterization of optogenetic tools for signaling control?
- Will new devices for better light delivery and interfacing with biological systems be developed?
- How do we implement optogenetics in therapeutic applications?

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References

1 Santos, S.D. *et al.* (2007) Growth factor-induced MAPK network topology shapes Erk response determining PC-12 cell fate. *Nat. Cell Biol.* 9, 324–330
2 Inoue, T. *et al.* (2005) An inducible translocation strategy to rapidly activate and inhibit small GTPase signaling pathways. *Nat. Methods* 2, 415–418
3 Karginov, A.V. *et al.* (2010) Engineered allosteric activation of kinases in living cells. *Nat. Biotechnol.* 28, 743–747
4 Deisseroth, K. (2011) Optogenetics. *Nat. Methods* 8, 26–29
5 Airan, R.D. *et al.* (2009) Temporally precise *in vivo* control of intracellular signalling. *Nature* 458, 1025–1029
6 Boyden, E.S. *et al.* (2005) Millisecond-timescale, genetically targeted optical control of neural activity. *Nat. Neurosci.* 8, 1263–1268
7 Zhang, F. *et al.* (2006) Channelrhodopsin-2 and optical control of excitable cells. *Nat. Methods* 3, 785–792
8 Nagel, G. *et al.* (2003) Channelrhodopsin-2, a directly light-gated cation-selective membrane channel. *Proc. Natl. Acad. Sci. U.S.A.* 100, 13940–13945
9 Karginov, A.V. *et al.* (2010) Light regulation of protein dimerization and kinase activity in living cells using photocaged rapamycin and engineered FKBP. *J. Am. Chem. Soc.* 133, 420–423
10 Liu, Q.Y. and Deiters, A. (2014) Optochemical control of deoxyoligonucleotide function via a nucleobase-caging approach. *Acc. Chem. Res.* 47, 45–55
11 Shestopalov, I.A. *et al.* (2007) Light-controlled gene silencing in zebrafish embryos. *Nat. Chem. Biol.* 3, 650–651
12 Arbely, E. *et al.* (2012) Photocontrol of tyrosine phosphorylation in mammalian cells via genetic encoding of photocaged tyrosine. *J. Am. Chem. Soc.* 134, 11912–11915
13 Gautier, A. *et al.* (2011) Light-activated kinases enable temporal dissection of signaling networks in living cells. *J. Am. Chem. Soc.* 133, 2124–2127
14 Nguyen, D.P. *et al.* (2014) Genetic encoding of photocaged cysteine allows photoactivation of TEV protease in live mammalian cells. *J. Am. Chem. Soc.* 136, 2240–2243
15 Tucker, C.L. (2012) Manipulating cellular processes using optical control of protein–protein interactions. *Prog. Brain Res.* 196, 95–117
16 Kim, B. and Lin, M.Z. (2013) Optobiology: optical control of biological processes via protein engineering. *Biochem. Soc. Trans.* 41, 1183–1188
17 Toettcher, J.E. *et al.* (2011) Light control of plasma membrane recruitment using the Phy–PIF system. *Methods Enzymol.* 497, 409–423
18 Zoltowski, B.D. and Gardner, K.H. (2011) Tripping the light fantastic: blue-light photoreceptors as examples of environmentally modulated protein–protein interactions. *Biochemistry* 50, 4–16
19 Tischer, D. and Weiner, O.D. (2014) Illuminating cell signalling with optogenetic tools. *Nat. Rev. Mol. Cell Biol.* 15, 551–558
20 Wu, Y.I. *et al.* (2009) A genetically encoded photoactivatable Rac controls the motility of living cells. *Nature* 461, 104–108
21 Yazawa, M. *et al.* (2009) Induction of protein–protein interactions in live cells using light. *Nat. Biotechnol.* 27, 941–945
22 Strickland, D. *et al.* (2010) Rationally improving LOV domain-based photoswitches. *Nat. Methods* 7, 623–626
23 Levskaya, A. *et al.* (2009) Spatiotemporal control of cell signalling using a light-switchable protein interaction. *Nature* 461, 997–1001
24 Shimizu-Sato, S. *et al.* (2002) A light-switchable gene promoter system. *Nat. Biotechnol.* 20, 1041–1044
25 Kennedy, M.J. *et al.* (2010) Rapid blue-light-mediated induction of protein interactions in living cells. *Nat. Methods* 7, 973–975

26 Crefcoeur, R.P. *et al.* (2013) Ultraviolet-B-mediated induction of protein–protein interactions in mammalian cells. *Nat. Commun.* 4, 1779
27 Chen, D. *et al.* (2013) A light-triggered protein secretion system. *J. Cell Biol.* 201, 631–640
28 Zhou, X.X. *et al.* (2012) Optical control of protein activity by fluorescent protein domains. *Science* 338, 810–814
29 Kent, K.P. and Boxer, S.G. (2011) Light-activated reassembly of split green fluorescent protein. *J. Am. Chem. Soc.* 133, 4046–4052
30 Do, K. and Boxer, S.G. (2013) GFP variants with alternative beta-strands and their application as light-driven protease sensors: a tale of two tails. *J. Am. Chem. Soc.* 135, 10226–10229
31 Strickland, D. *et al.* (2012) TULIPs: tunable, light-controlled interacting protein tags for cell biology. *Nat. Methods* 9, 379–384
32 Toettcher, J.E. *et al.* (2013) Using optogenetics to interrogate the dynamic control of signal transmission by the Ras/Erk module. *Cell* 155, 1422–1434
33 Zhang, K. *et al.* (2014) Light-mediated kinetic control reveals the temporal effect of the Raf/MEK/ERK pathway in PC12 cell neurite outgrowth. *PLoS ONE* 9, e92917
34 Bugaj, L.J. *et al.* (2013) Optogenetic protein clustering and signaling activation in mammalian cells. *Nat. Methods* 10, 249–252
35 Wend, S. *et al.* (2014) Optogenetic control of protein kinase activity in mammalian cells. *ACS Synth. Biol.* 3, 280–285
36 Vanhaesebroeck, B. *et al.* (2010) The emerging mechanisms of isoform-specific PI3K signalling. *Nat. Rev. Mol. Cell Biol.* 11, 329–341
37 Cantley, L.C. (2002) The phosphoinositide 3-kinase pathway. *Science* 296, 1655–1657
38 Toettcher, J.E. *et al.* (2011) Light-based feedback for controlling intracellular signaling dynamics. *Nat. Methods* 8, 837–839
39 Kakumoto, T. and Nakata, T. (2013) Optogenetic control of PIP3: PIP3 is sufficient to induce the actin-based active part of growth cones and is regulated via endocytosis. *PLoS ONE* 8, e70861
40 Idevall-Hagren, O. *et al.* (2012) Optogenetic control of phosphoinositide metabolism. *Proc. Natl. Acad. Sci. U.S.A.* 109, E2316–E2323
41 Kim, N. *et al.* (2014) Spatiotemporal control of fibroblast growth factor receptor signals by blue light. *Chem. Biol.* 21, 903–912
42 Grusch, M. *et al.* (2014) Spatio-temporally precise activation of engineered receptor tyrosine kinases by light. *EMBO J.* 33, 1713–1726
43 Mills, E. *et al.* (2012) Engineering a photoactivated caspase-7 for rapid induction of apoptosis. *ACS Synth. Biol.* 1, 75–82
44 Nagaraj, S. *et al.* (2013) Programming membrane fusion and subsequent apoptosis into mammalian cells. *ACS Synth. Biol.* 2, 173–179
45 Tyszkiewicz, A.B. and Muir, T.W. (2008) Activation of protein splicing with light in yeast. *Nat. Methods* 5, 303–305
46 Lee, S. *et al.* (2014) Reversible protein inactivation by optogenetic trapping in cells. *Nat. Methods* 11, 633–636
47 Taslimi, A. *et al.* (2014) An optimized optogenetic clustering tool for probing protein interaction and function. *Nat. Commun.* 5, 4925
48 Jungbluth, M. *et al.* (2010) Targeted protein depletion in *Saccharomyces cerevisiae* by activation of a bidirectional degron. *BMC Syst. Biol.* 4, 176
49 Renicke, C. *et al.* (2013) A LOV2 domain-based optogenetic tool to control protein degradation and cellular function. *Chem. Biol.* 20, 619–626
50 Bonger, K.M. *et al.* (2014) General method for regulating protein stability with light. *ACS Chem. Biol.* 9, 111–115
51 Rivera, V.M. *et al.* (2000) Regulation of protein secretion through controlled aggregation in the endoplasmic reticulum. *Science* 287, 826–830
52 Hughes, R.M. *et al.* (2012) Light-mediated control of DNA transcription in yeast. *Methods* 58, 385–391
53 Liu, H. *et al.* (2012) Optogenetic control of transcription in zebrafish. *PLoS ONE* 7, e50738
54 Motta-Mena, L.B. *et al.* (2014) An optogenetic gene expression system with rapid activation and deactivation kinetics. *Nat. Chem. Biol.* 10, 196–202
55 Polstein, L.R. and Gersbach, C.A. (2012) Light-inducible spatiotemporal control of gene activation by customizable zinc finger transcription factors. *J. Am. Chem. Soc.* 134, 16480–16483
56 Jullien, N. *et al.* (2003) Regulation of Cre recombinase by ligand-induced complementation of inactive fragments. *Nucleic Acids Res.* 31, e131
57 Boulina, M. *et al.* (2013) Live imaging of multicolor-labeled cells in *Drosophila*. *Development* 140, 1605–1613
58 Müller, K. *et al.* (2014) Optogenetics for gene expression in mammalian cells. *Biol. Chem.* Published online August 2, 2014. (http://dx.doi.org/10.1515/hsz-2014-0199)
59 Raftopoulou, M. and Hall, A. (2004) Cell migration: Rho GTPases lead the way. *Dev. Biol.* 265, 23–32
60 Gaj, T. *et al.* (2013) ZFN, TALEN, and CRISPR/Cas-based methods for genome engineering. *Trends Biotechnol.* 31, 397–405
61 Konermann, S. *et al.* (2013) Optical control of mammalian endogenous transcription and epigenetic states. *Nature* 500, 472–476
62 Lim, W.A. (2010) Designing customized cell signalling circuits. *Nat. Rev. Mol. Cell Biol.* 11, 393–403
63 Chow, B.Y. and Boyden, E.S. (2013) Optogenetics and translational medicine. *Sci. Transl. Med.* 5, 177ps5
64 Ni, M. *et al.* (1999) Binding of phytochrome B to its nuclear signalling partner PIF3 is reversibly induced by light. *Nature* 400, 781–784
65 Ni, M. *et al.* (1998) PIF3, a phytochrome-interacting factor necessary for normal photoinduced signal transduction, is a novel basic helix–loop–helix protein. *Cell* 95, 657–667
66 Khanna, R. *et al.* (2004) A novel molecular recognition motif necessary for targeting photoactivated phytochrome signaling to specific basic helix–loop–helix transcription factors. *Plant Cell* 16, 3033–3044
67 Liu, H. *et al.* (2008) Photoexcited CRY2 interacts with CIB1 to regulate transcription and floral initiation in *Arabidopsis*. *Science* 322, 1535–1539
68 Brautigam, C.A. *et al.* (2004) Structure of the photolyase-like domain of cryptochrome 1 from *Arabidopsis thaliana*. *Proc. Natl. Acad. Sci. U.S.A.* 101, 12142–12147
69 Yu, X. *et al.* (2010) The cryptochrome blue light receptors. *Arabidopsis Book* 8, e0135
70 Huala, E. *et al.* (1997) *Arabidopsis* NPH1: a protein kinase with a putative redox-sensing domain. *Science* 278, 2120–2123
71 Harper, S.M. *et al.* (2003) Structural basis of a phototropin light switch. *Science* 301, 1541–1544
72 Nihongaki, Y. *et al.* (2014) Genetically engineered photoinducible homodimerization system with improved dimer-forming efficiency. *ACS Chem. Biol.* 9, 617–621
73 Zoltowski, B.D. *et al.* (2009) Mechanism-based tuning of a LOV domain photoreceptor. *Nat. Chem. Biol.* 5, 827–834
74 Rizzini, L. *et al.* (2011) Perception of UV-B by the *Arabidopsis* UVR8 protein. *Science* 332, 103–106
75 Heijde, M. and Ulm, R. (2013) Reversion of the *Arabidopsis* UV-B photoreceptor UVR8 to the homodimeric ground state. *Proc. Natl. Acad. Sci. U.S.A.* 110, 1113–1118
76 Wu, D. *et al.* (2012) Structural basis of ultraviolet-B perception by UVR8. *Nature* 484, 214–219
77 Christie, J.M. *et al.* (2012) Plant UVR8 photoreceptor senses UV-B by tryptophan-mediated disruption of cross-dimer salt bridges. *Science* 335, 1492–1496
78 Rockwell, N.C. *et al.* (2006) Phytochrome structure and signaling mechanisms. *Annu. Rev. Plant Biol.* 57, 837–858
79 Wagner, J.R. *et al.* (2005) A light-sensing knot revealed by the structure of the chromophore-binding domain of phytochrome. *Nature* 438, 325–331